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Mr. Stephen Johnson, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building, 1101 -A
1200 Pennsylvania Ave., N.W.
Washington, DC 20460

Subject: Public Comments on the HPV Challenge Program Test Plan for Cobalt Salts of C8 to C13 Carboxylic Acids Including Neodecanoic Acid, Cobalt Salt (CASRN 27253-31-2), Fatty Acids, C9-C13 Neo, Cobalt Salts (CASRN 68955-83-9) and Hexanoic Acid, 2-Ethyl, Cobalt Salt (CASRN 136-52-7) by Members of the Metal Carboxylates Coalition (OM Group, Inc., The Shepherd Chemical Company and Troy Corporation).

The following comments on the HPV Challenge Program test plan for cobalt salts of C8 to C13 carboxylic acids by members of the Metal Carboxylates Coalition (OM Group, Inc., The Shepherd Chemical Company and Troy Corporation) are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

In our August 15, 2006 submission, we requested that EPA reopen the comment period for the metal carboxylates test plans, since, as a result of breaking up the category, the numbers of animals to be used has greatly increased and there are a number of serious scientific and animal welfare concerns that need to be addressed. These are the first of the comments that we will be submitting on the new individual test plans.

The sponsoring companies are proposing to conduct: an acute oral LD_{50} test for neodecanoic acid, cobalt salt; a combined repeated dose test with repro/developmental screen, OECD 422, for neodecanoic acid, cobalt salt; *in vivo* micronucleus tests for neodecanoic acid, cobalt salt and fatty acids, C9-C13 neo, cobalt salts and an acute fish toxicity test for neodecanoic acid. If conducted, these tests will cause the suffering and death of approximately 1,000 animals.

This test plan violates the following terms of the October 1999 agreement among the EPA, industry, and health, animal protection, and environmental organizations, as well as the December 2000 *Federal Register* notice reconfirming that agreement:

2. Participants shall maximize the use of existing and scientifically adequate data to minimize further testing.

3. Participants shall maximize the use of scientifically appropriate categories of related chemicals and structure activity relationships.

5. Participants are encouraged to use in vitro genetic toxicity testing to generate any needed genetic toxicity screening data, unless known chemical properties preclude its use.

Cobalt carboxylates are used as oxidative polymerization catalysts in many industries. Other uses include oxygen scavenger plastics and as adhesion promoters in tire manufacturing. Hexanoic acid, 2-ethyl, cobalt salt is used in paint driers, polyester initiators, and petrochemical catalysts. Neodecanoic acid, cobalt salt is used as a rubber adhesion promoter and plastic degradant.

The sponsoring companies note that metal carboxylates readily dissociate into free metal and free acid. The proportion of dissociated salt is dependent on the pH, and the dissociation constant (pKa) is the pH at which 50% dissociation occurs. The pKa values for the three category members as determined in studies conducted by the Metal Carboxylates Coalition are reported to be: 6.41 for hexanoic acid, 2-ethyl, cobalt salt; 6.52 for neodecanoic acid, cobalt salt; and 5.96 for fatty acids, C9-Cl3 neo, cobalt salts. These values indicate that complete dissociation will occur at the physiologically relevant pH of the mammalian stomach (pH 1.2). The sponsoring companies conclude therefore, that when administered orally, the toxicity of these metal carboxylates is due to the independent action of the respective acid and the free cobalt ion. As a result, mammalian toxicity data for the free acids and free metal ion, or its simple metal salts, can serve as surrogate data for that of the respective metal carboxylates. In support of this conclusion, the work of Stopford, et al. (2003)[1] is cited to show that cobalt chloride is similar to, or more bioavailable than, the corresponding cobalt carboxylate salts, thus making the chloride a conservative surrogate in estimating bioavailability and toxicity of the dissociated metal ion.

An acute oral LD_{50} test is proposed for neodecanoic acid, cobalt salt as a representative of the category. Although existing acute mammalian oral toxicity data is summarized for one category member, hexanoic acid, 2-ethyl, cobalt salt, no justification is offered as to why this endpoint for the other category members cannot be satisfied by read-across from this existing data. Reducing testing by this means is the rationale for establishing categories of related chemicals to begin with. In addition, existing data is summarized for: 2-ethylhexanoic acid; neodecanoic acid; fatty acids, C9-Cl3 neo; and cobalt (II) chloride – all of the three category members' dissociation products or, in the case of cobalt (II), the simple metal salt. The theoretical discussion of metal carboxylates dissociation presented in the test plan and summarized above clearly shows that, under the conditions of the proposed test (i.e. oral administration), existing data for the dissociation products fully characterize the toxicity of the three metal carboxylates in this category. No justification is offered for proposing a test which the sponsoring companies have convincingly argued is unnecessary.

Further, the OECD guidelines which the proposed test would follow are not specified. It is crucial to note that OECD 401 has been phased out in favor of OECD 425 and that the EPA now recommends the use of *in vitro* cell toxicity tests to establish the starting dose for acute toxicity tests (http://www.epa.gov/oppt/chemrtk/toxprtow.htm) in order to further reduce the number of animals used when an acute mammalian test is perceived to be necessary.

A combined repeated dose test with repro/developmental screen, OECD 422, is proposed for neodecanoic acid, cobalt salt as a representative of the category. As in the case of the proposed acute oral LD_{50} test, existing repeated dose toxicity data are summarized for all dissociation products of the three category members and repro/developmental data is summarized for 2-ethylhexanoic acid, neodecanoic acid and cobalt (II) chloride. Again, under the conditions of the proposed test, existing data for the dissociation products, along with category read-across for the C9-C13 neo, cobalt salts repro/developmental endpoints, satisfy the data requirements for the three metal carboxylates in this category, and the proposed test is therefore not required. Because the Metal Carboxylates Coalition submitted its original test plan in 2003, the sponsoring companies may be unaware that a similar approach, using existing data on dissociation products, was subsequently endorsed by the EPA and all stakeholders in 2004 for E. I. du Pont de Nemours & Company's test plan for triisopropylborate, a compound which breaks down to isopropanol and boric acid in water (see http://www.epa.gov/oppt/chemrtk/triprobt/c14841tc.htm). This approach has been used in a number of other test plans as well in which compounds dissociate at low pH and the toxicity data on the dissociation products has been used to meet the SIDS requirements.

In vivo micronucleus tests are proposed for neodecanoic acid, cobalt salt and fatty acids, C9-C13 neo, cobalt salts. As was the case for the proposed acute oral LD_{50} test, although existing chromosomal aberration data are summarized for one category member, hexanoic acid, 2-ethyl, cobalt salt, no justification is offered as to why this endpoint for neodecanoic acid, cobalt salt cannot be satisfied by read-across from these existing data. Once again, existing chromosomal aberration data are summarized for all of the three category members' dissociation products, thereby fully satisfying all data requirements for this endpoint. The sponsoring companies note mixed results in an *in vitro* chromosomal aberration test for fatty acids, C9-C13 neo; however, the proposed *in vivo* test for fatty acids, C9-C13 neo, cobalt salts clearly contradicts the principles laid out for the HPV Program in both the EPA's October 1999 letter to chemical sponsors and its December 2000 *Federal Register* notice on the program, which state that *in vivo* genotoxicity testing should be conducted only when known chemical properties preclude the use of *in vitro* testing. An *in vitro* chromosomal aberration test, OECD 473, should be conducted for fatty acids, C9-C13 neo, cobalt salts – per the *Federal Register* instructions – rather than the *in vivo* micronucleus test which will cause the suffering and death of 80 animals.

A fish acute toxicity test is proposed for neodecanoic acid, cobalt salt. No reliable ecotoxicity data for aquatic plants or invertebrates exist for any of the three members of this category. The fish test is intended to show whether exposure to these metal carboxylates will result in large-scale fish death thereby predicting economic loss and ecologic damage. If this exposure kills the food on which fish subsist, it could deplete fish populations even without direct fish toxicity. Since the toxicity of these metal carboxylates to aquatic plants and invertebrates is still unknown, tests on fish are premature. In addition, ECOSAR and non-animal ecotoxicity tests, such as the DarT test[2] and TETRATOX test[3] should be considered. In those cases for which fish acute toxicity tests are still perceived to be required, ECVAM's Ecotoxicology Task Force recently published an evaluation of a fish acute threshold (step-down) test concept with the potential to reduce the number of fish used in ecotoxicity testing by 53.6%-71.2%.[4]

In summary, while the sponsoring companies summarize existing data for members of this category and their dissociation products and present a convincing theoretical argument for the use of dissociation product data to serve as surrogates for those of the respective metal carboxylates, they nevertheless fail to use this analysis to minimize animal testing as specified by the EPA in both the October 1999 letter to chemical sponsors and the December 2000 *Federal Register* notice on the HPV program. Instead, the sponsoring companies propose additional testing for three mammalian toxicity endpoints without justification. In addition, the two proposed *in vivo* genotoxicity tests clearly contradict the EPA's instructions that *in vivo* genotoxicity testing should be conducted only when known chemical properties preclude the use of *in vitro* testing. We urge the sponsoring companies and the EPA to reject these proposed tests as well as to consider the applicability of the suggested alternatives to the fish acute toxicity test.

Sincerely,

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Research & Investigations

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[1] Stopford W., Turner J, Cappellini D, and Brock T. 2003. Bioaccessibility testing of cobalt compounds. J. Environ. Monit. 5(4): 675-680.
[2] Nagel, R. 2002. DarT: the embryo test with the zebrafish *Danio rerio*: A general model in ecotoxicology and toxicology. *ALTEX* 19 (Suppl. 1), 38-48.
[3] Schultz, T.W. 1997. TETRATOX *Tetrahymena pyriformis* population growth impairment endpoint: A surrogate for fish lethality. *Toxicological Methods* 7, 289-309.
[4] Jerama, S., et al. 2005. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. *Regulatory Toxicology and Pharmacology* 42, 218–224.